Effects of phencyclidine on schedule-controlled responding following neurotoxic lesions of the striatum

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Abstract

The effects of phencyclidine on an operant task were evaluated prior to and after neurotoxic lesions of the striatum in rats. Subjects were trained to respond on a fixed-interval 90-second schedule for water presentation. The degree to which phencyclidine disrupted responding was first evaluated (dose range 1.0–4.0 mg/kg). The subjects were then divided into three matched groups and received bilateral intraventricular injections of 6-hydroxydopamine (6-OHDA) (100 µg), kainic acid (0.25 µg), or vehicle delivered stereotaxically. 6-OHDA was used to destroy the presynaptic neurons of the nigro-striatal pathway and kainic acid was employed to destroy the postsynaptic neurons whose cell bodies are located in the striatum. Following recovery, the phencyclidine dose-response curve was repeated in the fixed-interval paradigm. It was observed that 6-OHDA-induced damage resulted in a rightward shift of the dose-response curve indicating tolerance to phencyclidine and produced a significant GABA depletion. Kainic acid-induced damage resulted in a leftward shift in the dose-response curve indicating sensitivity to the schedule-disruptive effects of phencyclidine and produced a significant GABA depletion. The vehicle-treated rats exhibited no shift in their sensitivity to phencyclidine. These observations indicate that the effects of phencyclidine are mediated, at least in part, by striatal dopaminergic neurons.

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Introduction

Phencyclidine is an arylcyclohexylamine compound that has received much investigative attention due to the complex spectrum of behavior it induces and its complicated interactions with the central nervous system. Phencyclidine administration may mimic pharmacological properties similar to those of stimulants, depressants, hallucinogens and analgesic agents depending upon the dose and species tested. Neurochemical evidence has linked the actions of phencyclidine with the dopaminergic, noradrenergic, GABAergic, enkephalinergic and serotonergic systems. Phencyclidine was originally developed for use in humans as an anesthetic in 1959, but was soon restricted due its psychotomimetic effects which include prolonged thought and visual disturbances and agitation. Illicit phencyclidine administration in humans has resulted in long lasting schizophrenic-like behavior that has led many investigators to support this as a pharmacological model of schizophrenia (Meltzer and Stahl, 1976; Javitt and Zukin, 1991; Jentsch and Roth, 1999; Farber, 2003; Svenningsson et al., 2003).

Striatal function is largely influenced by both glutamatergic and dopaminergic afferent input. Evidence from several studies indicates that phencyclidine acts as a glutamatergic antagonist at the N-methyl-D-aspartate (NMDA) receptor, and as an indirect dopaminergic agonist via blocked dopamine reuptake into nerve terminals (French et al., 1991). Investigators have shown that mice having reduced NMDA receptor expression display behavioral abnormalities similar to that which is seen in pharmacologically induced schizophrenia and these behaviors can be attenuated by antipsychotic drugs such as haloperidol and clozapine (Mohn et al., 1999). Furthermore, neonatal dopamine lesions in rats produce high rates of phencyclidine-induced locomotor activity, demonstrating enhanced sensitivity to NMDA antagonists. This increase in locomotion caused by phencyclidine is ameliorated following administration of olanzapine, an atypical antipsychotic (Moy and Breese, 2002).

Behavioral studies have found that locomotor stimulation by phencyclidine is mediated by dopamine mechanisms (French and Vantini, 1984; French et al., 1985) whereas electrophysiological studies have shown phencyclidine’s action on dopamine neurons is positively correlated with its affinity as a non-competitive NMDA receptor antagonist (French and Ceci, 1990; Svensson, 2000). Neurochemically, phencyclidine has been shown to cause the release of presynaptic dopamine in vitro preparations (Doherty et al., 1980; Vickroy and Johnson, 1982; Dwoskin et al., 1992; Balla et al., 2001) and block the reuptake of released dopamine (Garey and Heath, 1976; Smith et al., 1977; Nishijima et al., 1996). It has also been demonstrated that non-competitive NMDA antagonists, such as phencyclidine and N-[1-(2-thienyl)cyclohexyl]piperidine (TCP), stimulate tyrosine hydroxylase activity in vitro (Vickroy and Johnson, 1981; Mateu et al., 2000). However, in vivo studies indicate that phencyclidine may actually inhibit tyrosine hydroxylase activity (Hitzemann et al., 1973; Doherty et al., 1980).

Some behavioral components of the phencyclidine profile have been directly linked to phencyclidine’s action on the central dopaminergic system. Phencyclidine causes ipsilateral rotation in rats with unilateral lesions of the nigrostriatal dopamine pathway (Finnegan et al., 1976; Fessler et al., 1979; Mele et al., 1997). This phencyclidine-induced rotation can be antagonized by pretreatment with haloperidol, pimozide (Finnegan et al., 1976) or reserpine (Fessler et al., 1979). Phencyclidine administration to rodents produces an array of unconditioned behaviors which resemble those of other psychomotor stimulants including increased motor activity, stereotypic sniffing, cage circling, and head weaving (Schlemmer et al., 1978; Sturgeon et al., 1981; Castellani and Adams, 1981; McCullough and Salamone, 1992; Svenningsson et al., 2003; Takahata and Moghaddam, 2003). These stereotypic behaviors and increased motor activity can also be antagonized by pimozide.
(Schlemmer et al., 1978) as well as haloperidol (Sturgeon et al., 1981; White et al., 1995) and clozapine (Sturgeon et al., 1981; Sams-Dodd, 1996; Abekawa et al., 2003). It has also been demonstrated that phencyclidine-induced increases in motor activity are significantly attenuated following 6-hydroxydopamine lesions of the mesolimbic dopamine system (French and Vantini, 1984; French et al., 1985).

In schedule-controlled behavior paradigms, low doses of phencyclidine have been shown to increase low rates of responding and to decrease high rates of responding, whereas, higher doses of phencyclidine produce a decrease in responding independent of response rate (Wenger and Dews, 1976; Brady et al., 1980; Segal et al., 1981; Wagner et al., 1984a,b; Sanger and Jackson, 1989). These rate-dependent actions of phencyclidine have been demonstrated in mice (Wenger and Dews, 1976) and rats (Segal et al., 1981; Wagner et al., 1984a,b; Sanger and Jackson, 1989). The present studies were designed to further elucidate the relationship between phencyclidine and the dopamine system by evaluating the degree to which the schedule-disruptive effects of phencyclidine are altered following neurotoxic lesions of the striatum.

**Materials and methods**

**Subjects**

The animals were experimentally naive male Sprague-Dawley rats (Taconic, Germantown, NY) weighing 250-300 grams at the start of the experiment. Rats were housed in standard suspended metal cages in a colony room with a 12h/12h light/dark cycle (lights on at 8:00 AM). Animals had free access to food and water. The animals used in the behavioral testing procedure had free access to food and were water-deprived 23.5 h/day.

**Apparatus**

Experimental sessions were conducted in an operant box (51 × 33 × 48 cm) enclosed in a ventilated sound-attenuating chamber with an observation window. The manipulandum was a Gerbrands (Model G6312) lever mounted 12 cm above the floor and 6 cm below a GE1813 session light. A water dipper, which delivered 0.1 ml tap water, was located 4.0 cm to the right of the lever and 0.5 cm above the floor. Programming and recording equipment were located in an adjacent room. The experimental session was 15 min in duration. The rats were allowed 15 min access to water beginning 15 min after the session had ended. Sessions were conducted seven days/week between 2–6 PM.

**Behavioral testing procedure**

The animals were first trained to lever press for water reinforcement on a continuous-reinforcement schedule. When the animals were reliably responding, they were shifted to a fixed-interval 90 seconds (FI-90) schedule of reinforcement where the first response after 90 seconds resulted in water presentation. When responding on the FI-90 schedule had become stable (less than 10% variation in session response rate over a 3-day period), phencyclidine in varying doses or 0.9% saline was administered IP 20 min prior to the session. The phencyclidine or saline was administered no more
often than every third day and only after a stable baseline was obtained. The doses of phencyclidine were varied in a random manner until a complete dose-response curve was obtained with partial replication. The animals were then matched for their initial sensitivity to phencyclidine and divided into three groups. They were rehydrated for one week and then surgery was performed as described below. The rats were allowed to recover one week following surgery and then returned to fluid deprivation. They were returned to the FI schedules until baseline rates were reestablished. Post-treatment dose-response curves for phencyclidine were determined in a procedure identical to the pre-lesion dose response curves.

Surgery and neurochemistry

Stereotaxic surgeries were accomplished using a Kopf stereotaxic instrument under sodium pentobarbital anesthesia (50 mg/kg) administered IP. The coordinates for the lateral ventricle were 2.0 mm posterior to bregma, 3.0 mm off the midline suture and 5.5 mm below the dura with the incisor bar set at +5.0 mm. 6-Hydroxydopamine (6-OHDA) was administered at two doses. Rats were first pretreated with 50 mg/kg of pargyline dissolved in 0.9% saline 45 minutes prior to surgery. The animals received bilateral intraventricular injections of either 100 μg or 200 μg of 6-OHDA (salt weight) dissolved in 5 µl of 0.1% ascorbate through a Hamilton syringe at a rate of 1.0 µl/min. Two groups of animals received bilateral injections of kainic acid (0.25 μg or 0.5 μg/5 µl) dissolved in 0.9% saline. A third group was subdivided into two groups of 4, with each group receiving bilateral injections of the same volume of either 0.1% ascorbate or 0.9% saline. Following one month, these animals were sacrificed, striata dissected and assayed for dopamine and serotonin content using high performance liquid chromatography (Wagner et al., 1984a,b).

The surgery performed on the animals from the behavioral study was done as described above. There were eight animals in each of three treatment groups. The dose of 6-OHDA was 100 μg/side and the dose of kainic acid was 0.25 μg/side. The control animals were subdivided into two groups. The first was treated with intraventricular saline (0.9%), while the second was treated with intraventricular ascorbate (0.1%).

Sixteen additional behaviorally naive rats received unilateral intraventricular injections of either 6-OHDA (100 μg) or kainic acid (0.25) as above. Following one month, the animals were sacrificed and the striata were dissected. Gamma amino-butyric acid (GABA) levels were determined according to the following procedure. The tissue was weighed and homogenized in 10 vol of 0.4 N perchloric acid and centrifuged at 14,800 rpm for 20 minutes at 4 °C. O-pthaldehyde (Sigma) was added to an equal amount of supernatant (40 μls). This reactant forms a substituted isoindole which is identified through fluorescence detection. Following precisely two minutes of reaction time, the fluorescent product was injected into an open loop of a Waters Gradient HPLC across a spherical C-18 column in a volume of 40 μls. Two buffers were simultaneously used with the gradient changing throughout a 45-minute period. The first buffer was composed of 0.094M sodium phosphate dibasic, 0.05M sodium acetate, 1% methanol and 1% tetrahydrofuran in deionized water. The second buffer was a 65% methanol solution of deionized water. At the time of injection, the percentage of the organic buffer was 0%, which was gradually increased to 100% and decreased back to 0% over a 45-minute period. Fluorescence was detected by a Waters fluorescent detector, and quantification was accomplished with external standards.
Statistics

Pretreatment dose response curves were analyzed using a repeated measures ANOVA for repeated measures. Comparison of pre- versus post-treatment dose response curves was accomplished with 2-way ANOVA for matched subjects. The Scheffé test was used to conduct the post hoc analyses. The nonparametric Sign test was used for neurochemical comparisons of the unilaterally lesioned animals. Quarter-life values at the highest doses were not determined if the total number of responses made within a session were less than 20.

Results

Neurochemistry

The control subjects which were treated with intraventricular ascorbate or intraventricular saline were not significantly different from one another on any neurochemical measure; therefore, the values were pooled for the control groups.

Neurochemical assay of the 6-OHDA-treated animals revealed a significant depletion of dopamine at both doses tested in comparison to control animals (F (2,24) = 37.7; p < .0001). Dopamine levels for the animals treated with 100 µg/side of 6-OHDA were significantly reduced to approximately 24% of the control values (2.2614 ± 0.6276 µg/g and 9.3330 ± 0.8882 µg/g for lesion and control, respectively). Analysis of the animals treated with the higher dose of 6-OHDA (200 µg/side) revealed an even greater depletion of striatal dopamine to approximately 15% of control values (1.3690 ± 0.3954 µg/g and 9.3330 ± 0.8882 µg/g for lesion and control, respectively). Two animals died following surgery at this dose of 6-OHDA. Post hoc analyses revealed that the two groups treated with 6-OHDA were not significantly different from one another; therefore, the 100 µg/side dose was chosen for the behaviorally trained animals. Neither dose of 6-OHDA resulted in any alteration in striatal serotonin levels (p > .05).

Intraventricular administration of kainic acid resulted in no alteration in striatal dopamine levels at either dose tested (0.25 or 0.5 µg/side). These two treatments also had no significant effect on striatal serotonin levels. There were no deaths following either of these treatment regimens; therefore, the higher dose of kainic acid was used in the behavioral study.

Within-subject comparison indicated that unilateral intraventricular administration of 100 µg of 6-OHDA resulted in approximately a 17% loss of striatal GABA content (0.3047 ± 0.0217 µg/g and 0.3667 ± 0.0458 µg/g for lesion and control respectively; p < .05, Sign test). Unilateral kainic acid (0.5 µg) resulted in a 34% depletion of striatal GABA content in comparison to the intact side (0.2422 ± 0.0415 µg/g and 0.3675 ± 0.0487 µg/g for lesion and control, respectively; p < .05, Sign test).

Behavioral

Three rats died following surgery before completion of the post-lesion dose response curves. Data from these rats were excluded from all analyses. However, these subjects were included when the animals were first matched for their initial sensitivity to phencyclidine’s effects. Due to this fact, there is a slight discrepancy in the pre-lesion baseline performance between the three treatment groups with regard to response rate; however, this discrepancy was not statistically significant (Fig. 1A.) Similarly,
comparison of pre-lesion number of reinforcers between groups revealed no significant differences (Fig. 2A). For the 21 remaining rats, the pre-lesion dose response curve for phencyclidine on response rate revealed a mild biphasic effect (Fig. 3A). The average response rate following saline treatment was 9 responses/min. Administration of 1.0 mg/kg of phencyclidine produced a modest increase in the average response rate. The response rate following 2.0 mg/kg of phencyclidine was nearly equal to that obtained

![Comparison of pre-lesion and post-lesion response rate/minute between Control, 6-OHDA and KA groups. Vertical bars = SEM.](image1)

![Comparison of pre-lesion and post-lesion number of reinforcers between Control, 6-OHDA and KA groups. Vertical bars = SEM.](image2)
Fig. 3. Pretreatment dose response curve of the means of A) response rate, B) number of reinforcers/session and C) quarter-life values for all animals (N = 21) collapsed across groups. Vertical bars = SEM. (* Scheffe post hoc analyses, p < .05; in comparison to saline baseline performance).
during saline treatment. Finally, at a dose of 4.0 mg/kg, the response rate dropped significantly below baseline levels (Scheffe test; p < .05). The ED$_{50}$ for phencyclidine was approximately 3.6 mg/kg.

The effect of phencyclidine on the number of reinforcers received per session was also dose dependent (Fig. 3B). Following saline administration, the average number of reinforcers received was 10.2. There were minor decreases in the number of reinforcers/session at doses of 1.0 and 2.0 mg/kg. The average number of reinforcers received following 4.0 mg/kg significantly decreased (Scheffe test; p < .05). No tested dose of phencyclidine resulted in a 50% decrease in reinforcers/session.

The average quarter-life values (Fig. 3C) for the saline treatment (0.6) indicates that the FI schedule generated a pattern of responding that was typically scalloped, signifying that subjects made few responses in the early part of the interreinforcement interval and increased responding as the delivery of

![Graph](image_url)

Fig. 4. Pre- and post-treatment phencyclidine dose response for A) number of reinforcers/session and B) response rate for animals treated with 6-OHDA (N = 7). Vertical bars = SEM.
the reinforcer drew nearer. Phencyclidine at 1.0 and 2.0 mg/kg did not significantly disrupt this pattern of responding and generated quarter-life values comparable to those during saline sessions. The highest dose (4.0 mg/kg) generated an average quarter-life value of 0.22 which was significantly lower than that obtained following saline administration (Scheffe test; \( p < .05 \)).

Comparison of the pre- and post-treatment dose responses curves for the 6-OHDA-treated animals revealed alterations in both the number of reinforcers/session and response rate following the lesion. The shape of the dose response for the number of reinforcers/session did not change following the 6-OHDA treatment (Figs. 2B, 4); however, there was a slight shift to the right in the post-treatment function. A similar effect on response rate was seen in the post-treatment dose-response function; the curve was significantly shifted to the right with no change in the shape of the function (\( F (1,6) = 6.12; p < .05 \)).

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**Fig. 5.** Pre- and post-treatment phencyclidine dose response for A) number of reinforcers/session and B) response rate for animals treated with kainic acid (\( N = 7 \)). Vertical bars = SEM.
Finally, there was also a significant effect of dose on response rate in the post-treatment dose-response curve ($F(2,12) = 10.73; p < .01$; Figs. 1B, 4).

Comparison of the pre- and post-treatment dose-response curves for the subjects treated with kainic acid revealed a dose-dependent decrease in the number of reinforcers/session following the kainic acid treatment similar to the effect seen prior to the lesion (Figs. 2B, 5). A repeated measures ANOVA revealed this dose effect to be significant ($F(2,12) = 4.00; p < .05$). The slope of this function was steeper following the kainic acid lesion and the function was shifted to the left (by a factor of 2); however this effect was not significant. In these same animals, phencyclidine administration produced a significant dose-dependent decrease in response rate ($F(2,12) = 10.71; p < .01$). The post-lesion dose

![Fig. 6. Pre- and post-treatment phencyclidine dose response for A) number of reinforcers/session and B) response rate for sham operated animals (N = 7). Vertical bars = SEM.](image-url)
response function of phencyclidine on response rate was also shifted to the left indicating enhanced sensitivity to phencyclidine’s schedule disruptive effects (Figs. 1B, 5).

Analysis of the post-treatment data obtained from the sham-operated animals demonstrated no significant changes on either measure, number of reinforcers or response rate, following the sham operation in comparison to the pretreatment data (Fig. 6). The post-treatment effect of dose on the number of reinforcers received was significant (F (2,12) = 6.6; p < .05) as well as the effect of phencyclidine dose on response rate (F (2,12) = 11.14; p < .01). There was no significant treatment effect or treatment by dose interaction (Figs. 1B, 2B).

Discussion

The topography of the disruption of schedule-controlled responding by phencyclidine reported in this paper concurs with previous findings in the rat and monkey (Byrd, 1982; Wagner et al., 1984a,b). Administration of low doses of phencyclidine produced increases in response rate, while higher doses produced decreases. The effect of phencyclidine on the number of reinforcements received within a session was dose-dependent with each increasing dose resulting in a greater decrease in the average number of water presentations. The measure of reinforcements proved to be less sensitive to pharmacological disruption than response rate in FI schedules indicated by differences in estimated ED₅₀. Phencyclidine also produced rate-dependent alterations illustrated by decreasing quarter-life values at higher doses.

The intraventricular administration of kainic acid appeared to affect those cells which were intrinsic to the striatum. There was a significant loss of striatal GABA content (34%) in response to this treatment, while striatal dopamine and serotonin were unaffected. The subjects treated with kainic acid exhibited a slightly enhanced sensitivity to phencyclidine’s effects in comparison to the prelesion dose response. The shift in the dose response was small, however, and was substantial only at the highest dose tested. It is unclear why striatal cell loss would enhance phencyclidine’s behaviorally disruptive effects in the rat. In fact, other data would suggest that striatal kainate lesions may actually reduce phencyclidine’s behavioral effects. It has previously been shown that striatal kainic acid lesions attenuate the stereotypy that is induced by phencyclidine administration at high doses (Nabeshima et al., 1985). However, the drug dose as well as the behavioral indices in this study were different than those used in the present study.

The intraventricular administration of 6-hydroxydopamine resulted in a substantial reduction of striatal dopamine to 24% of control levels. This treatment had no effect on striatal serotonin. Following 6-OHDA treatment, there was also a small but significant reduction in striatal GABA. The nonselective loss of striatal GABA may be due to a nonspecific effect of 6-OHDA at this high dose.

The post-treatment dose response curve for these animals indicated tolerance to the disruptive effects of phencyclidine on both response rate and the number of reinforcers per session. It is likely that the tolerance to phencyclidine’s effects following 6-OHDA may be attributable to a decrease in phencyclidine’s dopaminergic action following such an extensive lesion of the nigrostriatal system. It appears from evidence gathered from in vitro studies that phencyclidine exerts its dopaminergic action through presynaptic mechanisms by inducing the release of dopamine (Doherty et al., 1980; Vickroy and Johnson, 1982; Dwoskin et al., 1992; Balla et al., 2001) as well as inhibiting its reuptake (Garey and Heath, 1976; Smith et al., 1977; Nishijima et al., 1996). Electrophysiological evidence also implicates a
presynaptic site of action for the mediation of phencyclidine’s dopaminergic effects. It has been demonstrated that direct application of phencyclidine to striatal neurons results in the inhibition of spontaneous activity of these cells. This inhibition is blocked by pretreatment with neuroleptics and also attenuated by pretreatment with 6-OHDA or reserpine (Johnson et al., 1984). Given that phencyclidine’s dopaminergic actions are presynaptically mediated, it is possible that the loss of dopaminergic input to the striatum following 6-OHDA treatment may result in a lessened response of this system to phencyclidine. This decreased drug action may be the underlying phenomenon mediating the tolerance to phencyclidine exhibited by the 6-OHDA-treated animals in this study.

The tolerance to phencyclidine’s behaviorally disruptive effects following 6-OHDA may also be the result of a loss of phencyclidine receptors in the striatum. It has been reported that 6-OHDA lesions of the nucleus accumbens results in a decreased number of phencyclidine binding sites (French et al., 1985). This observation may indicate that at least some of the phencyclidine binding sites are located presynaptically on the dopaminergic neurons. In the present study, the 6-OHDA treatment may have resulted in a loss of phencyclidine binding sites in the striatum and, thereby, attenuated phencyclidine’s action at the receptor level. However, it has not been demonstrated that this same phenomenon which occurs in the mesolimbic system also occurs in the nigrostriatal system and it is therefore, with caution, that this alternative interpretation is offered. It is clear, however, that at least some of phencyclidine’s behaviorally disruptive actions are mediated, in part, through the nigrostriatal dopamine system. These data suggest that this system warrants future investigation in order for the varied and complex actions of phencyclidine to be further elucidated.

Conclusion

In conclusion, 6-OHDA-induced damage resulted in a rightward shift of the dose-response curve indicating tolerance to phencyclidine and caused a significant depletion of striatal dopamine and gamma-aminobutyric acid (GABA). Kainic acid-induced damage resulted in a leftward shift in the dose-response curve indicating sensitivity to the schedule-disruptive effects of phencyclidine and produced a significant GABA depletion. The vehicle-treated rats exhibited no shift in their sensitivity to phencyclidine. These observations indicate that the effects of phencyclidine are mediated, at least in part, by striatal dopaminergic neurons.

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